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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

MEYER et al. Art Unit: 1645

Application No.: 09/284,233 Examiner: V. Portner

Filed: July 28, 1999 Attorney Dkt. No.: 100564/09008

For: HELICOBACTER PYLORI LIVE VACCINE

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Thomas F. Meyer, declare as follows:

1. I am an Applicant of the above-identified patent application.

2. Under my direction and control, a prophylactic live vaccine approach was used to protect against H. pylori infection. Recombinant Salmonella were employed as a carrier of various H. pylori immunogenic proteins. Several bacterial constructs with differing antigens were shown to mediate protection against a H. pylori challenge in a mouse model.

3. The following protocol was used for the prophylactic vaccination.

On day 0, six to eight week old female specific pathogen free BALB/c mice were immunised intra-gastrically with 200 µl (100 mM $NaHCO_3$) of a bacteria suspension containing 10^9 colony-forming units (cfu) of *Salmonella typhimurium* SL3261::YZ222 Δ*thy*A expressing the respective antigens. Antigens were coded on stabillsed plasmids employing the plasmid-coded *thy*A gene as a means of balanced lethality to complement the chromosomally deleted *thy*A. Plasmids allowed for expression of the

various antigens driven by one out of three respective expression signals (P_{phoP}, P_{nirB}, P_{T7}). S e attached Exhibit 1, illustrating combinations of express signals and various antigens used for prophylactic vaccination. On day 28, mice were challenged intragastrically with a total of 10^9 cfu of H. pylori strain P76 (strep^R) suspended in a volume of 100 µl (100 mM $NaHCO_3$). At day 49, mice were sacrificed, and half of the stomachs was plated on GC agar supplemented with 8% serum and 10 µg/ml streptomycin, allowing growth of H. pylori P76 (strep^R), and the number of streptomycin-resistant H. pylori cfu was determined. See attached Exhibit 2, illustrating the recovery H. pylori from stomachs of immunized and challenged mice.

4. The prophylactic live vaccination approach proved protective immunity using urease A and B subunits under control of a P_{T7} expression signal as a positive control. The respective plasmid was termed pYZ97 and was described before (Gomez-Duarte et al., 1998). Protection was determined by cfu counting, and a mock control construct (CREA1412) did not mediate protection. Naively infected mice also were highly susceptible to a H. pylori challenge.

5. In general, the results were independent of the expression signal used. As determined by cfu counting, HylB (CREA1396 and CREA1402), citrate synthase homolog, (CREA1398 and CREA1404), and GroEL (strains CREA1467 and CREA1468) were protective. Thus, specific protection due to recombinant H. pylori antigens expressed by a live vector is achieved.

6. The Helicobacter immunogens employed raised protection levels similar or even better than Urease A and B subunits according to cfu counting.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may

jeopardize the validity of the above-referenced application or any patent issuing thereon.

Date: 23. Juli 2001

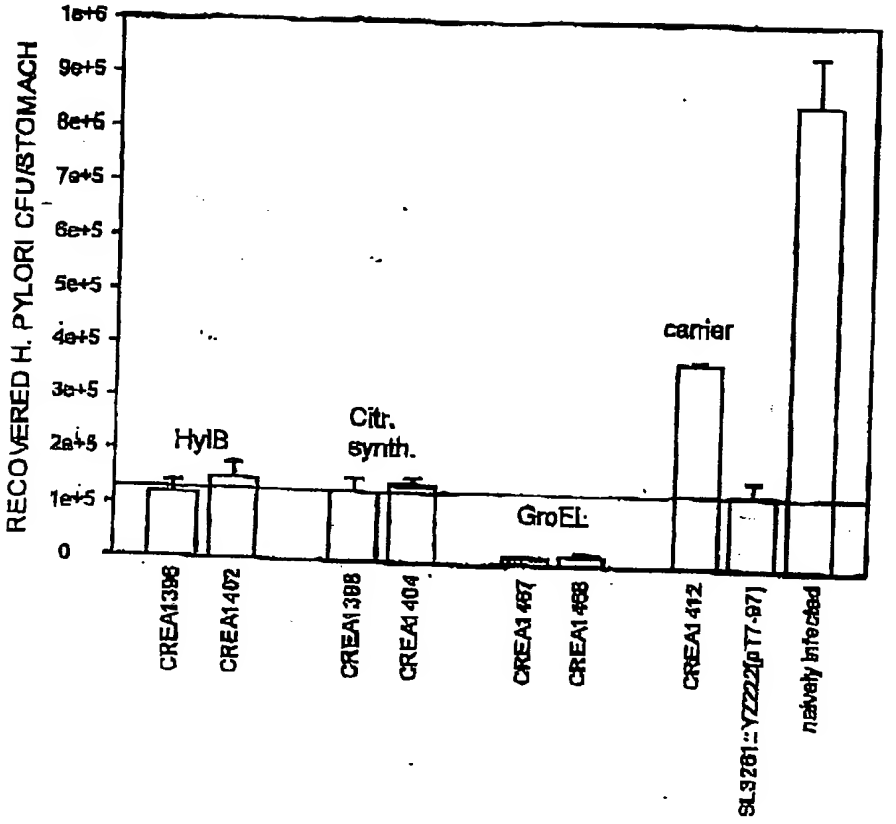
Thomas F. Meyer

Exhibit 1:

Combinations of expression signals and various antigens used for prophylactic vaccination

Construct	Antigen	Expression signal
CREA1396	HylB	P_{T7}
CREA1398	Citrate synthase homolog	P_{T7}
CREA1402	HylB	P_{nirB}
CREA1404	Citrate synthase homolog	P_{nirB}
CREA1412	none	none
CREA1467	GroEL	P_{nirB}
CREA1468	GroEL	P_{phoP}
SL3261:: YZ222 Δ*thy*A [pT7-97]	Urease A subunit Urease B subunit	P_{T7} internal promoter

Exhibit 2



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